Local IgA subclass alterations in ulcerative colitis and Crohn's disease of the colon

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SUMMARY The subclass distribution of IgA producing cells was determined by paired immuno-fluorescence staining in colonic specimens from 10 patients with ulcerative colitis and eight with Crohn's disease. Compared with normal colonic mucosa, the percentage of IgA1 immunocytes showed a striking increase in both disorders. The proportion of mucosal IgA1 cells was significantly higher ($p<0.05$) in ulcerative colitis (median, 71·2%) than in Crohn colitis (median, 56·9%). Within each category of specimens no significant differences were noted between luminal and basal mucosal zones. The submucosal proportion of IgA1 cells was, however, significantly higher than the mucosal one in both ulcerative colitis (median, 89·1%; $p<0.002$) and Crohn colitis (median, 91·8%; $p<0.005$). The mucosal shift towards IgA1 production paralleled the magnitude of the submucosal IgA1 cell proportion in individual tissue samples. Taken together with the previously reported dramatic increase of local IgG production (particularly observed in the submucosa and basal parts of the mucosa), our findings indicated that there is an influx and/or proliferation of B cells representing systemic secondary immunity in the lesions of both diseases.

The numerical increase of mucosal IgG and IgA producing immunocytes in the order of 30 and two times, respectively, suggests that a pronounced and disproportionate local B cell response takes place in ulcerative colitis and Crohn's disease compared with the predominant normal colonic secretory IgA response.[1] Very little is known about the antibody specificities of the disease associated B cells.[1] In ulcerative colitis there is relatively more local synthesis of the IgG1 subclass than in Crohn colitis; this has been shown both immunohistochemically[2] and by quantification of spontaneous IgG secretion from isolated intestinal lymphoid cells.[3] Such a disparity between the two diseases is of pathogenetic interest. Both IgG and IgA antibodies may show subclass restriction to different antigens,[4-10] and further dissection of the local B cell responses is necessary to identify microbial components involved in inflammatory bowel disease.

The four IgG subclasses show functional differences which may be of biological importance.[11]

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The two subclasses of IgA (IgA1 and IgA2)[12-14] that can be distinguished immunologically are, however, apparently functionally identical except that IgA1 is susceptible to a variety of IgA-specific microbial proteases.[15 16] Moreover, the increased proportion of IgA2 in secretions compared with serum,[17] and the relatively high frequency of IgA2 producing immuno-cytes in the distal gut mucosa,[18] suggest that the two subclasses are subjected to different immuno-regulatory mechanisms.

In this study we wanted to see if altered local immune regulation in inflammatory bowel disease is reflected by a shift in the mucosal distribution of IgA subclass-producing immunocytes.

Methods

PATIENTS AND MATERIALS
Ten patients with ulcerative colitis, four men and six women (median age, 39 years; range, 20 to 49 years) showing a median disease duration of three years (range, three weeks to 17 years) were included in the study along with eight patients with Crohn's disease of the colon, three men and five women (median age,

28 years; range, 15 to 64 years) showing a median disease duration of eight years (range, two to 21 years). All patients had lesions throughout the entire length of the colon. Eight of those with ulcerative colitis and three with Crohn colitis were under treatment with corticosteroids. One Crohn patient was additionally treated with azathioprine and one received this drug only.

The final diagnosis was based on clinical features in combination with endoscopic findings or inspection of surgically resected material and on histopathological examination. Mucosal specimens were obtained at colonic resection (carried out because of severe disease activity or to avoid development of cancer in longstanding colitis) or by endoscopic suction biopsy at follow up examinations. All tissue specimens were from either the transverse or sigmoid colon. The mucosal IgG subclass response pattern in the same patients has been reported previously.[2]

IMMUNOHISTOCHEMISTRY

Tissue specimens were prepared as thin slices which were extracted in cold phosphate buffered isotonic saline to remove diffusible, extracellular proteins before ethanol fixation and paraffin embedding.[19] Three serial sections were cut at 6 μm from each tissue block; one was stained with haematoxylin and eosin for histologic examination and two were subjected to paired immunofluorescence staining (Fig. 1). The latter sections were first incubated for 20 hours at room temperature with murine monoclonal antibody to IgA1 (Clone 69-11·4; ascites, 1:5000) or IgA2 (Clone 16-512-H5; ascites, 1:10000) produced and tested for specificity as described elsewhere.[20] The second incubation step included a mixture of rhodamine B sulphonyl chloride labelled rabbit antihuman IgA and fluorescein isothiocyanate labelled rabbit antimouse IgG. The characteristics and working concentrations of these conjugates have been described elsewhere.[18] They were applied for 20 hours at room temperature. To avoid fading of fluorescein emission the sections were, after the final wash, mounted in buffered (pH 8) polyvinyl alcohol containing paraphenylenediamine.[21]

MICROSCOPY AND CELL COUNTING

Fluorescent cells with a distinctly stained cytoplasm were observed in a Leitz Ortoplan microscope equipped with X25 and X40 immersion objectives, an X10 ocular, and a Ploem-type vertical illuminator with interference filters for selective observation of fluorescein (green) and rhodamine (red) emission (Figs 2 and 3). Cell counting was done by the same observer throughout the study. The intra and interobserver reproducibility of this enumeration method has been reported previously.[18]
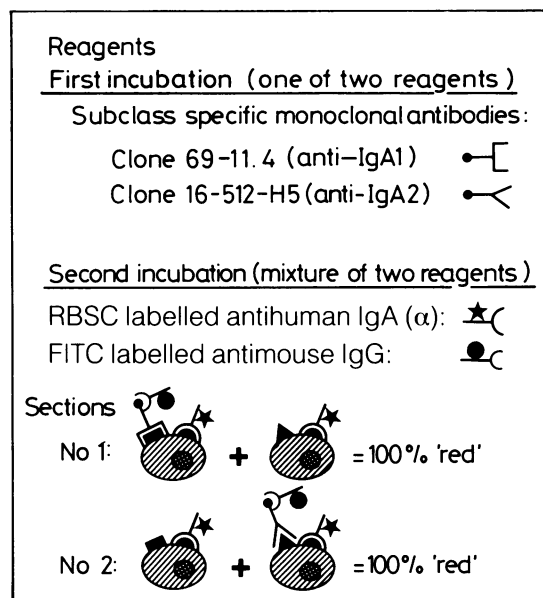

Fig. 1   Schematic illustration of paired immunofluorescence staining method and enumeration of IgA subclass producing cells. The clone numbers refer to the codes of antibody suppliers. Rhodamine (RBSC) labelled antihuman IgA was α chain specific ('red'); fluorescein (FITC) labelled antimouse IgG was used to label indirectly the two monoclonal antibodies.

To obtain an indication of disease associated subclass alterations, our enumerations were expressed as immunocyte ratios for individual specimens (which thereby all had the same statistical weight). The counts were based on mucosal areas with dense infiltrates of Ig-producing immunocytes which usually extended into the submucosa, especially in Crohn colitis.

Areas with ulcers and fissures were avoided because of persistent background staining and relative scarcity of IgA producing cells. While IgG producing cells were located mainly in such areas and in the deeper layer of the mucosa (and also in the submucosa)[22] IgA immunocytes were abundantly present throughout the mucosa and particularly in the luminal part.

In all but one of the paired adjacent sections of each disease category, the lamina propria could be divided into a luminal and a basal zone in which the IgA cells were counted separately. The submucosa was lacking in three specimens of ulcerative colitis and in one of Crohn colitis; for the other samples IgA cells were counted separately also in the submucosa.

For each tissue specimens an average of 605 red (class specific) cells (range, 136 to 1474) were examined for concomitant green (subclass specific)
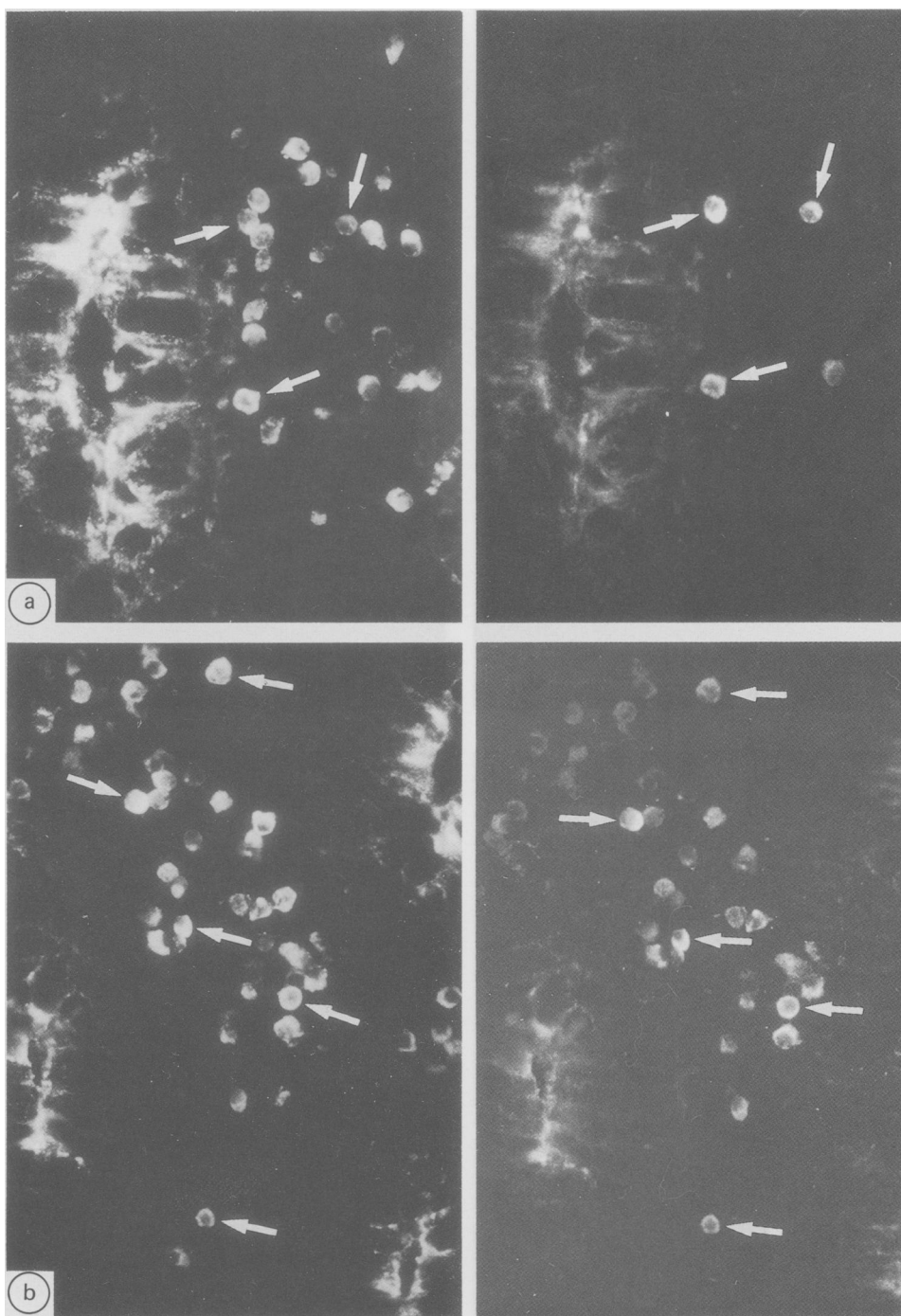
Fig. 2   (a) *Paired immunofluorescence staining for IgA (left panel, rhodamine) and IgA1 subclass (right panel, fluorescein) in the same field from section of normal colonic mucosa. (b), Similar staining for IgA (left) and IgA2 (right) in adjacent section. Note relatively large proportion of IgA2-producing cells and uptake of both subclasses in crypt epithelium. Examples of identical cells in paired pictures are indicated by arrows.*
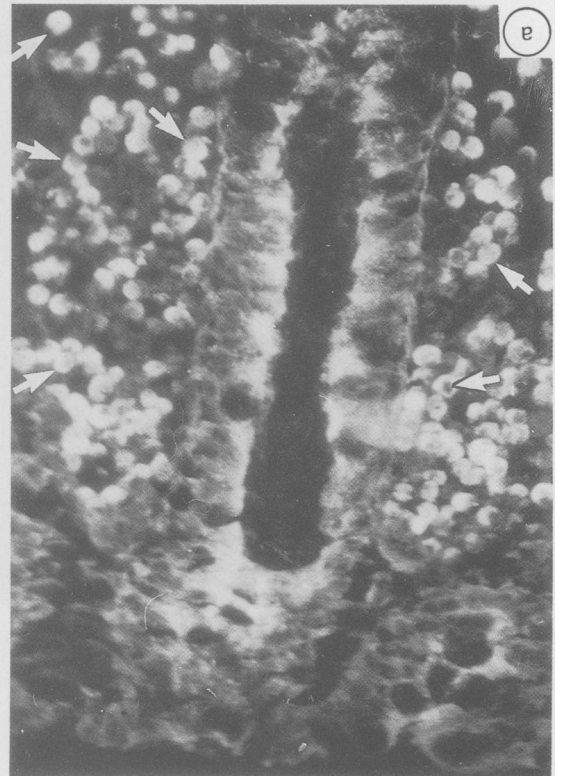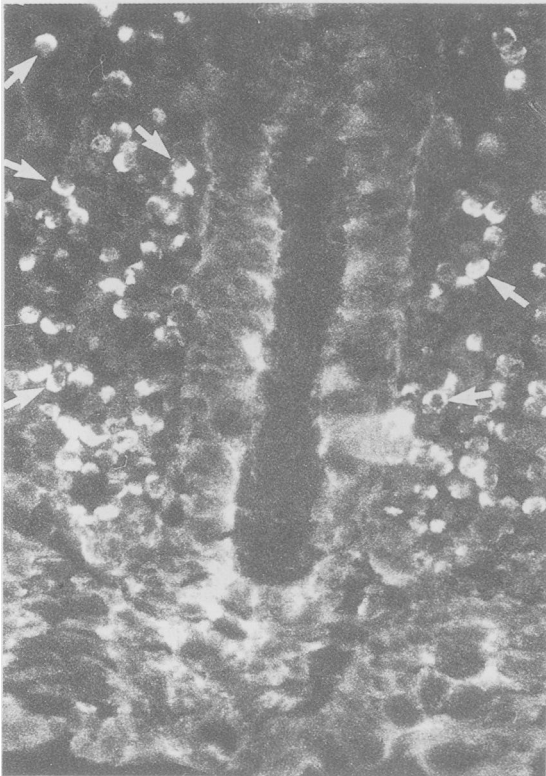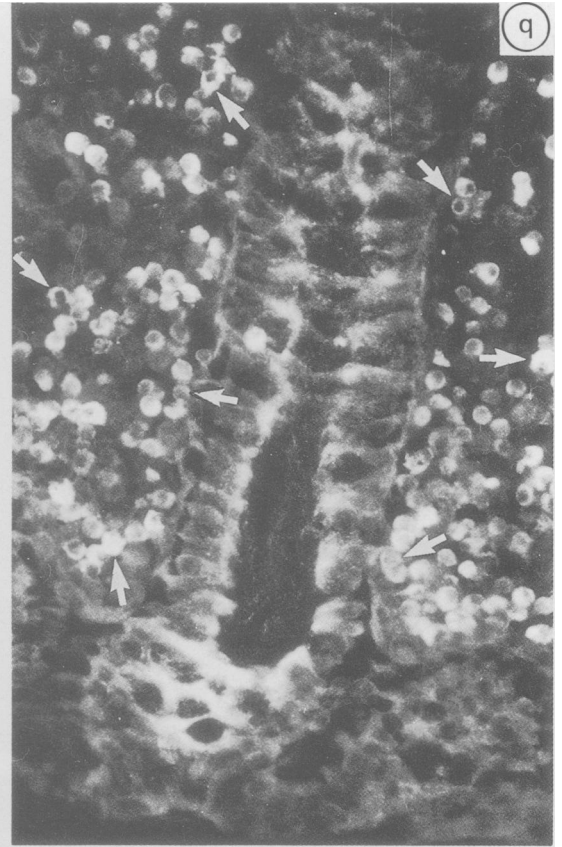
fluorescence. The proportion of subclass positive immunocytes was then calculated in relation to the total number of IgA producing cells present in the evaluated area of each section (Fig. 1). The sum of the IgA1 and IgA2 percentages in the two adjacent sections was usually close to 100% (range, 96%–107%) and so was also the sum of the subclass medians calculated for each disease category (Fig. 4).

STATISTICAL ANALYSIS
Differences between proportions of the two subclasses were calculated by Wilcoxon's two-tailed test for unpaired samples. The relations between mucosal and submucosal IgA1 percentages were determined by analysis of covariance.

Results

Figure 4 shows the percentage distribution of mucosal and submucosal IgA1 and IgA2 cells in the two disorders as compared with that previously observed in normal colonic mucosa.[18] A striking and highly significant increase of the mucosal IgA1 percentage was found both in ulcerative colitis (p<0·02) and in Crohn colitis (p<0·015). Moreover, the mucosal IgA1 cell proportion was significantly higher (p<0·05) in ulcerative colitis (median, 71·2%) than in Crohn colitis (median, 56·9%). Conversely the IgA2 cell proportion was significantly higher (p<0·05) in the latter (median, 43·1%) than in the former (median, 31·5%) disorder.

Neither disease showed significant differences between luminal and basal mucosal zones. The proportion of IgA1 cells in the submucosa, however, was in both ulcerative colitis and Crohn colitis significantly higher (p<0·002 and p<0·005, respectively) than in the mucosa. In Crohn colitis the submucosal IgA1 percentage was 91·8% and in ulcerative colitis 89·1%. In individual tissue specimens a strikingly parallel contribution of IgA1 producing cells was observed in the mucosa and submucosa (Fig. 5).

Discussion

This is the first immunohistochemical study on the subclass distribution of IgA producing immunocytes in colonic specimens from patients with ulcerative colitis and Crohn's disease of the colon. We found in both disorders a marked increase of the mucosal IgA1 to IgA2 cell ratio compared with the immunocyte distribution in normal colonic mucosa.[18] This shift to local IgA1 production was significantly larger in ulcerative than in Crohn colitis.

Immunohistochemical studies have unequivocally shown a striking predominance (80-90%) of IgA containing immunocytes in normal intestinal mucosa.[23-25] These cells produce mainly dimers (or larger polymers) as evidenced by concurrent staining for J chain along with cytoplasmic affinity for secretory component (SC) *in vitro*[26 27] and by spontaneous release in cultures.[28 29] Local production of polymeric IgA (pIgA) is the basis for secretory immunity because epithelial SC acts as a pIgA receptor, thereby facilitating external translocation of secretory IgA.[30] In a recent study of the normal intestinal IgA subclass distribution, however, we found a striking heterogeneity in that IgA1 cells dominated (77%) proximally whereas large bowel mucosa contained mainly (59%) IgA2 cells.[18] Although both pIgA subclasses can bind to SC,[31] this observation indicated regional differences in the regulation of intestinal immunity.

Published data show large discrepancies with regard to the mucosal distribution of Ig-producing cells in ulcerative colitis and Crohn's disease.[22 32-35] Various methodological problems may explain these differences as discussed elsewhere.[25] Studies based on cultures of intestinal lymphoid cells have confirmed our original immunohistochemical findings of a strikingly increased local IgG production in both disorders.[3 35-38] Conflicting culture results have been published with regard to IgA.[29 36-38] Spontaneous IgA secretion *in vitro* is difficult to interpret in quantitative terms, however, because of uncontrolled dilutional effects caused by a disproportionate increase of the IgA cell fraction.

Our quantitative immunohistochemical studies of intestinal immunocytes have been based on a 'tissue unit' which represents a 6 μm thick and 500 μm wide block of the mucosa from the muscularis mucosae to the gut lumen.[22] On this basis we reported that the total number of IgA producing cells showed approximately a two fold increase in both ulcerative and Crohn colitis.[34] In the present study we found that this increase was mainly explained by IgA1 producing cells. Our observation is in keeping with recently published results based on spontaneous IgA release from dispersed lymphoid cells obtained from

Fig. 3 (a) *Paired immunofluorescence staining for IgA (left panel, rhodamine) and IgA1 subclass (right panel, fluorescein) in the same field from section of colonic mucosa from patient with Crohn's disease. (b) Similar staining for IgA (left) and IgA2 (right) in adjacent section. Note high immunocyte density and predominance of IgA1 producing cells; the latter subclass also predominates in crypt and surface epithelium (gut lumen at the top). Examples of identical cells in paired pictures are indicated by arrows.*
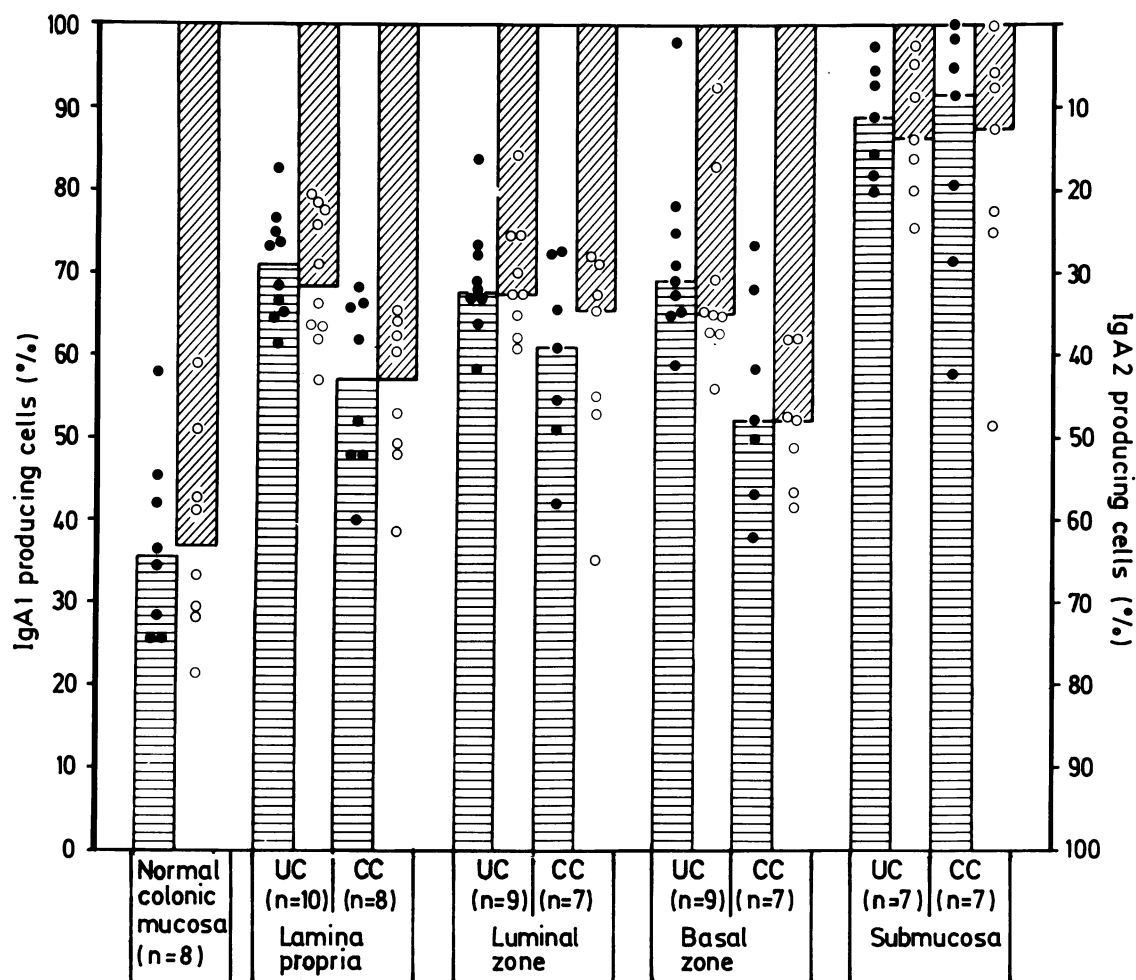
Fig. 4   *Scatter diagram of percentage distribution of IgA1 (●) and IgA2 (○) cells in normal colonic mucosa[18] and in diseased colonic lamina propria (total mucosa and luminal and basal zones) and submucosa from patients with ulcerative colitis (UC) and Crohn's disease of the colon (CC). The median percentages for IgA1 and IgA2 cells are indicated by differently hatched columns.*

the gut wall down to the musculature.[29] It is difficult to know what extent submucosal cells were included in the cultures, and the results were indistinguishable for the two diseases. Our findings showed that the percentage of IgA1 cells in the mucosa was significantly related to that in the submucosa which, however, was always much higher than the former. These results paralleled our observations of a shift towards local IgA monomer production in inflammatory bowel disease as determined immuno-histochemically by staining for J chain expression and cytoplasmic SC affinity.[26] Again, these findings were in keeping with observations on dispersed cells from the lesions.[29 39]

Altogether, the markedly increased number of J

chain negative IgG producing mucosal cells[1 27] and the shift towards local production of monomeric IgA[27] and the IgA1 subclass, show that the secretory immune system is partially replaced by B cells belonging to mature memory clones compatible with a systemic type of immunity. These cells may originate directly from the circulation and migrate from the basal parts of the mucosa into the lamina propria and the submucosa. The submucosal infiltrate contains Ig-producing cells which in terms of class and subclass proportions match those present in bone marrow,[40 41] peripheral lymph nodes and tonsils.[41 42] Alternatively, IgA1 cells may develop within the tissue lesions as a result of heavy chain switching from the abundant precursors of the IgG1
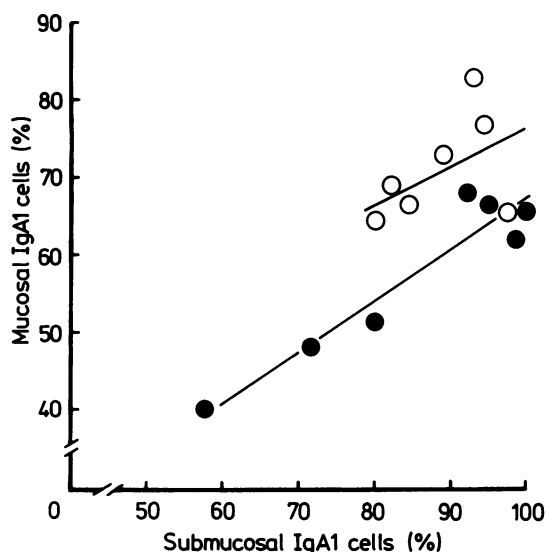
Fig. 5. *Scatter diagram with regression lines of IgA1 immunocyte percentage in mucosa on that of submucosa in patients with ulcerative colitis (UC, o) versus Crohn's disease of the colon (CC, ●). Analysis of covariance did not reveal any established difference between the series-specific deviations from regression or between the corresponding regression coefficients. The regression lines could therefore be assumed to be parallel, with a common regression coefficient of +0·625% increase in mucosal IgA1 per unit increase in submucosal content (p<0·001). The 95% confidence limits of the common slope were estimated at + 0·352 and +0·898. The adjusted mean of the UC series (66·3%) was above that of the CC group (62·2%; p<0·005).*

isotype, as recently reported for human myeloma cells.[43 44]

An additional possibility is that the disease associated immunocytes originate in solitary lymphoid nodules within the mucosa. We have recently found that there is an IgG-dominated population of plasma cells with repressed J chain expression associated with these structures and with the Peyer's patches.[45] These lymphoid sites are collectively considered responsible for generation of the precursor cells that 'home' to secretory sites and end up mainly as J chain positive pIgA producers.[46] Retention and terminal maturation to plasma cells *in situ*, therefore, seem to involve only relatively mature B cell clones. It is possible that this precursor fraction is dramatically increased in the diseased mucosa because of massive exposure to luminal antigens and mitogens. The shift to mucosal IgA1 production was more pronounced in ulcerative colitis than in Crohn's disease, which might reflect better preservation of mucosal integrity in the latter (areas of overt ulceration were avoided).

It is at present unknown whether the underlying immunoregulatory alterations are primary or secondary events in the pathogenesis of inflammatory bowel disease. A shift towards local production of monomeric IgA does not support secretory immunity, and there is some indication that secretory antibodies to lipotheichoic acid and bacterial lipopolysaccharides are preferentially carried by IgA2.[6 9] In addition, IgA1 is susceptible to IgA specific proteases which may occur in faeces.[47]

Nevertheless, it is questionable whether the observed alterations in local IgA production will compromise secretory immunity. In patients with Crohn's disease circulating IgA shows in fact some increase of the pIgA fraction and a tendency to a raised IgA2 proportion,[48] probably as a reflection of an intensified secretory immune response in the distal bowel mucosa. The concurrent pronounced local IgG production may be of much greater pathogenic significance as discussed elsewhere.[1] This possibility is supported by the recently reported differences between ulcerative colitis and Crohn colitis as to the subclass distribution of mucosal IgG immunocytes.[2]

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